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UPON POLYEMBRYONY AND ITS MORPHOLOGY IN OPUNTIA VULGARIS.

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(WITH PLATE XVI)

MANY cases of polyembryony are now known, occurring in widely separated groups. Braun, in his summary of the subject in 1860, recorded twenty-one cases in twelve families and thirteen genera, which number has been added to by others. Strasburger was the first to thoroughly investigate its morphological basis, and since his paper in 1878 important contributions have been made to the subject by several investigators, notably Dodel (1890), Overton (1891), Tretjakow (1895), Jeffrey (1895), and Hegelmaier (1897). The results of these works, in so far as they touch this subject, will be found summarized below. In the Cactaceæ, the only case of polyembryony hitherto known has been that of *Opuntia tortispina*, which Engelmann, in his Cactaceæ of Whipple's expedition figured (*pl. 23, fig. 4*) as having two embryos in one seed. This case was cited by Braun (p. 155, *pl. 5, figs. 18–20*), who also suggests that the four cotyledons which he had himself noticed in *Opuntia glaucophylla* may indicate a fusion of two embryos and hence polyembryony, though he points out that it may also be explained as fasciation. While studying the seedling stages in this family, I have found that *Opuntia vulgaris* is markedly polyembryonic, and I may here add that although I have worked over the seedlings of some seventy-five species in this family I have seen no other case of it.

The plants from which my seeds were taken have been growing and flowering luxuriantly for at least four years in the Botanic Garden of Smith College. They agree with the characters given in books for that species, but the source from which they came into the garden is unknown. They set seed every year in great abundance. When the seeds are planted, from many, perhaps a half, of them more than one seedling comes up, and there is the greatest variation in the number, size, and degrees of union with one another of these seedlings. This variety is best made evident by the *figs. 1* to *5*, typical cases drawn the natural size, and of course there are all stages between. I have not tried to follow them further, but what I have noticed seems to show that the larger of a set crowds out the others. It is now important to ascertain the morphological origin of this polyembryony.

The ovule of *Opuntia vulgaris* is at first amphitropous, but in its development it becomes elongated and bent, at the same time turning around in such a way that the funiculus makes a complete turn around it, so that finally it simulates a campylotropous condition (*fig. 6*). Its development in other species of this genus, together with the development of other species of other genera in this family, has been described by d'Hubert, and his account fits this species fairly well. Inside of the funiculus are the integuments, made up of three distinct layers of cells, and inside of these is a nucellus, which becomes absorbed, except for a small portion (at *x*, *fig. 6*), by the embryo as the seed ripens. Finally there is the distinct embryo sac, in which there forms after fertilization an abundant endosperm, which consists of protoplasts without cellulose walls, the whole of course absorbed by the growing embryo.

In ripe seeds one finds usually a large embryo nearly filling it, with others much smaller and pressed to one side. In half-ripe seeds one finds such a condition as is shown in *fig. 8*, where there is one larger embryo with several smaller ones, and usually the larger comes not from the micropylar extremity of the embryo sac, but from some point on its wall a little removed. In other cases one sees a single embryo springing from the micropylar end and one or more from the walls, as in *fig. 7*. Or again, though rarely, there is a single embryo at the micropylar end, as in *fig. 9*. There is a close resemblance between these figures and those given by Strasburger for *Citrus Aurantium* (*fig. 37*). If now these be examined in a still younger stage, it becomes clear that the embryos come from two different positions: first, from a rather irregular mass of tissue which lies at the micropylar end of the embryo sac, and extends thence along its wall; and second, directly from the wall itself. Both of these conditions are well shown in *fig. 10*. It is important to notice, however, that some cases seeming to belong to the latter category belong really to the former, as is illustrated by *fig. 15*, where the wall-standing embryos are shown to spring really from the mass at the micropylar end, but this is not the case with all of the wall-standing embryos, for sections show that in many cases, *i. e.*, *fig. 10*, these are entirely independent of that mass. It is probable that the irregular embryos come as a rule from the micropylar mass, while the regular ones are from the walls, for all I have seen in that position have regularly two cotyledons.

So far, in tracing backwards the origin of the polyembryony, everything is plain, and it is easy to find plenty of cases such as are here figured. At this stage, the entire distinctness of the micropylar mass from the nucellus beneath (see *figs. 10* and *11*) and its close resemblance to that described and figured by Jeffrey which he traced to its origin in a fertilized egg cell, would lead one to suppose that we have here a similar case; while in the wall-standing embryos, which are so sharply distinct from the nucellus (*fig. 11*) that an origin from nucellus seems excluded, only an origin from an endosperm cell would appear possible, a condition which is yet unknown.[1] But the earlier stages show that both of these suppositions are incorrect. In a great

[1] This was my own opinion in both cases at the time this paper was read before the

abundance of material which shows the ovules in all stages before and at the time of fertilization, and also all stages after that represented in *fig. 10*, I was able to find, after prolonged and careful search, only three cases which show the origin of the embryos, but happily they leave no doubt on the subject. I think this stage must be passed through very rapidly, and perhaps at night, when none of my material was collected.

In stages earlier than *fig. 10*, the pollen tube can usually be seen in the micropylar region of the nucellus, but an egg cell cannot be detected. Instead there regularly lies in its position a crumpled mass of protoplasm (*fig. 12, 14, pr.*), shrunken in my material by the weak alcohol used to preserve it. I think the egg cell disappears early in the development of the ovule, a point which will be cleared up by a complete study of the development of the embryo sac, now being made by one of my students. At all events there is certainly no egg cell present in any of my preparations at the period when the embryo building begins. The nucellus cells near the pollen tube are rich in contents, and in one case (*fig. 12*) I have found these beginning to bud out into the embryo sac. That these represent the beginning of the formation of the micropylar mass, I think there can be no question. It is precisely in this way that the adventitious embryos originate in Funkia and other cases, as described so fully and clearly by Strasburger. In another case (*fig. 13*) there are present not only the richly protoplasmic nucellus cells near the pollen tube, here, however, not budding, but also other nucellus cells a little removed and separated by a space filled with cells rather poor in contents; and these cells with rich contents are also distinctly budding out (*fig. 13, x*). Now these, I believe there can be no doubt, are the beginning of the wall-standing embryos; it is quite probable that at first they are continuous with those near the pollen tube, but are removed from them by the growth in length of the embryo sac, which at this time is very rapid. At all events they are nucellus cells. The

Society for Plant Morphology and Physiology, Dec. 29, 1897. I had not then found the three cases next to be described, which prove the origin to be different.

third case I have found is represented in *fig. 14*, which is more advanced than *fig. 13*, and where the differentiation of the richly protoplasmic cells of the embryonic mass from the nearly empty cells beneath it is fairly sharp, but nevertheless the relations of the two kinds are still sufficiently close to show the origin of the one from the other. Another section of the same specimen shows a similar connection of the other mass of embryonic tissue with the nucellus.

From these cases it is clear that in this species the embryos of both positions arise from the nucellus, and in this respect agree with Funkia, Nothoscordum, Citrus, and others, the usual method.

A synopsis of the modes of origin of polyembryony is given by Tretjakow, which in synopsis, with the additions made by others, and excluding cases due to branching of the nucellus, union of two ovules, presence of two or more embryo sacs in one nucellus, etc., are as follows: From cells of nucellus near the micropyle, *Funkia* and others (Strasburger); from a second egg cell, *Santalum*, or from its doubling *Sinningia* (Strasburger); from synergids, *Mimosa Denhartii* (Guignard), *Iris Sibirica* (Dodel), and perhaps in *Lilium Martagon* (Overton); from the antipodals and synergids, *Allium odorum* (Tretjakow); from the branching of a mass of tissue derived from the fertilized egg cell (Jeffrey); finally, Hegelmaier points out the occurrence in *Allium odorum* of embryos from egg cell, synergids, antipodals and nucellus cells, all in the same species and individuals. There still remain as possible origins, endosperm and integument. In all known cases, except *Cœlebogyne*, the production of the new embryos takes place only after fertilization, or at all events after the entrance of the pollen tube.

A question of great interest now arises as to the significance of polyembryony. Strasburger, though he mentions that it recalls apogamy, then recently discovered by Farlow, contents himself with referring to the extra embryos as "Adventivknospen" or "vegetative Adventivkeime." Pfeffer[2] has suggested that it is but a case of budding, and that the specific conditions

[2] Pflanzenphysiologie 1: 29. 1897.

in the embryo sac determining the form of the sexually produced embryo, give the same form to the adventitious embryos. Tretjakow suggests that the development from the antipodals represents apogamy, the antipodals being homologous with the vegetative cells of the prothallus, but this explanation will not apply to the origin from nucellus cells. Jeffrey has nothing to say on this point, while Hegelmaier concludes his paper thus:

> Die verhältnissmässige Seltenheit der dem Eiapparat entspringenden Polyembryonie bei *A. odorum* einerseits und das öftere gelegentliche Vorkommen dieser Form bei verschiedenen anderen Pflanzen andererseits könnte vielleicht zu der Auffassung führen, dass ihr Vorkommen bei unserer Pflanze überhaupt nicht in dieselbe Reihe mit dem Vorkommen der anderen Formen von Polyembryonie bei ihr zu stellen und ihr Zussammentreffen mit diesen anderen Formen mehr nur ein zufälliges sei. Aber mindestens für diese letzteren ist doch wohl die Annahme unabweisbar, das irgendwelche gemeinschaftlichen Ursachen vorhanden sein müssen, für deren Erkenntniss durch ein Spiel mit morphologischen Homologien nichts zu gewinnen sein würde.

In general for a new feature one of three origins may be supposed. First, it may be some incidental growth or functional condition. Here comes the explanation of budding, and Pfeffer's explanation of the assumption of the embryo form through specific qualities of the embryo sac. But polyembryony seems too distinct and elaborate a process to be thus explained. Second, it may be a relic of some older condition now disappearing. But its very different morphological origins are against this. Here comes its explanation as apogamy, but this does not explain the origin from the nucellus. Third, it may be the early stages in the development of something new. It can hardly yet be of any service to the plants, for many of the embryos are absorbed before the seeds are ripe, and in other cases usually but one develops, though perhaps one of the smaller may sometimes take the place of the leader if this is destroyed. Its origin in several distinct groups and by several distinct methods seems to imply that there is some virtue in the development of the extra embryos, and that their appearance is controlled by that influence, whatever it may be, which is much more powerful than mere morphological inertia, and which elsewhere forms new structures from the most different morphological origins. In its independent appearance in distinct groups it is comparable with the appearance of heterospory, but whether polyembryony like heterospory will lead to some higher condition remains to be seen, though we shall not see it.

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A fuller bibliography and references may be found in Tretjakow.

EXPLANATION OF PLATE XVI.

Figures all drawn with camera lucida, and the original drawings reduced one-half.

In all of the figures, *e. s.* = embryo sac ; *n.* = nucellus ; *in.* = the integuments collectively ; *i. i.* = inner integument ; *m.* = micropyle or micropylar opening through the nucellus ; *p.* = pollen tube ; *pr.* protoplasm of embryo sac.

FIGS. 1, 2, 3, 4, 5, each showing the embryos from a single seed, natural size.

FIG. 6. Median section through an ovule of *Opuntia vulgaris*, somewhat diagrammatized, six times natural size : *f* = funiculus ; *a* = attachment of funiculus to ovule ; *x* = portion of nucellus not absorbed when seed is ripe.

FIG. 7. Embryo sac showing three embryos, one from the micropylar end and two from the wall. × 18.

FIG. 8. Embryo sac showing one large embryo on the wall and several smaller from the micropylar end. × 18. Not a section, but a half seed laid open.

FIG. 9. Embryo sac showing a single embryo from the micropylar end. × 35.

FIG. 10. Embryo sac showing embryos on the wall together with the branching mass from the micropylar end. Nucellar cells drawn nearly exactly; those of embryos exact on the outside but only approximate in the interior. × 90.

FIG. 11. An embryo on the wall; to show the connection of cells of embryo to those of nucellus; a microtome section. × 90.

FIG. 12. Beginning of budding from nucellus cells near the micropyle. × 90.

FIG. 13. Cells of nucellus beginning to grow out at *x* into an embryo. × 90.

FIG. 14. Embryonic mass separating from the nucellus. The section is cut diagonally through the apex of the embryo sac. × 90.

FIG. 15. *a*, *b*. Two sections from one embryo sac to show that some apparently wall-embryos are really from the micropylar mass. × 18.